IN VIVO ACTIVITIES OF ETHANOLIC EXTRACT FROM *Endiandra kingiana* (LAURACEAE) AS POTENTIAL ANTI-DIABETIC AGENTS

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by

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DEDICATION

I would like to dedicate this dissertation to my parents and siblings for their support and encouragement throughout the journey.

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

°C	Degree Celsius
A1C	Estimated average glucose
ADP	Adenosine diphosphate
ALT	Alanine aminotransferase
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate-activated protein kinase
ARASC	Animal Research and Service Centre
AST	Aspartate aminotransferase
ATP	Adenosine tri-phosphate
BMI	Body mass index
BUN	Blood urea nitrogen
cm^2	Square centimetre
dl	Decilitre
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DPP-4	Dipeptidyl peptidase IV
EKEE	*E. Kingiana* bark ethanolic extract
ELISA	Enzyme-linked immunosorbent assay
et al.	Et alia/and others
FBG	Fasting blood glucose
g	Grams
GLUT	Glucose transporter
GSH	Reduced glutathione

H	Hours
HFD	High-fat diet
HLA	Human leukocyte antigen
HO	Hydroxyl radicals
HRP	Horseradish peroxidase
IACUC	Institutional Animal Care and Use Committee
IC50	Half-maximal inhibitory concentration
IDF	International Diabetes Federation
IL	Interleukin
IP	Intraperitoneal injection
IR	Insulin resistance
Ki	Inhibition constant
L	Litre
LFT	Liver function test
M	Molar
MDA	Malondialdehyde
mg	Milligram
MHC	Major histocompatibility complex
min	Minutes
mmHg	Millimetre of mercury
mmol	Millimole
MOH	Ministry of Health
NAD^+	Nicotinamide adenine dinucleotide
NGOs	Non-government organisation
NGSP	National glycohemoglobin standardisation program

NIDDM	Non-insulin-dependent diabetes mellitus
NIH	National Institute of Health
nm	Nanometre
NO	Nitric oxide
O_2^-	Superoxide anions
OGTT	Oral glucose tolerance test
OHA	Oral antihyperglycemic agents
$ONOO^-$	Peroxynitrite
RFT	Renal function test
ROS	Reactive oxygen species
S.D.	Standard deviation
SD	Sprague-Dawley
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic-pyruvic transaminase
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
T2DR	Type 2 diabetic rats
TAOC	Total antioxidant capacity
TCM	Traditional and Complimentary Medicine
TNF	Tumour necrosis factor
TOR	Target of rapamycin
U/L	Units per litre
umol	micromole
US	United States

USM	Universiti Sains Malaysia
WHO	World Health Organisation
β	Beta
μM	micromolar

AKTIVITI *IN VIVO* EKSTRAK ETANOL DAN KITARAN POLIKETIDA DARI *Endiandra kingiana* (LAURACEAE) SEBAGAI POTENSI AGEN ANTI-DIABETIK

ABSTRAK

Diabetes mellitus adalah salah satu penyakit mempunyai kelaziman yang tinggi dalam populasi dunia. Kekurangan dalam penghasilan insulin, kerbekesanan insulin atau kedua-duanya menyebabkan hiperglisemia. Pada tahun 2019, 4.2 juta kematian dilaporkan akibat diabetik. Hiperglisemia tidak terkawal mencetuskan komplikasi lain seperti retinopati, nefropati, kardiopati dan amputasi anggota badan. Oleh itu, salah satu pokok di jumpai di Malaysia dikenali sebagai *Endiandra kingiana* (pokok medang) dinilai. Ia mempunyai kesan perencatan α-glukosidas *in vitro* namun kekurangan bukti untuk aktiviti antidiabetik *in vivo*. Oleh itu, terdapar keperluan untuk menilai kesan anti-diabetik ekstrak etanol batang *E. kingiana* (EKEE) ke atas tikus diabetik jenis 2 (T2DR). Kajian terbahagi kepada akut (24 jam) dan subakut (28 hari). T2DR diaruh dengan kombinasi diet tinggi lemak (HFD) dan dos-rendah streptozotocin (STZ). Dos EKEE pada 250 mg/kg adalah efektif dalam menurunkan paras glukosa darah puasa (FBS) dalam kajian akut. Selanjutnya, kesan ini dikaji selama 28 hari dalam kajian sub-akut. Tiga puluh tikus Spague-Dawley (SD) jantan dibahagi kepada 4 kumpulan. Kumpulan 1 – normal, Kumpulan 2 – tikus diabetik-tidak terawat, Kumpulan 3 – tikus diabetik terawatt-EKEE (250 mg/kg) dan Kumpulan 4 – tikus diabetic terawat-metformin (300 mg/kg). FBS, berat badan, BMI dan SBP diukur setiap dua minggu. Di akhir kajian, RFT, LFT, profil lipid, glukagon, dan penanda stres oksidatif dinilai. EKEE tidak menurunkan FBS dengan ketara. EKEE juga tidak menghalang penurunan

berat badan dan BMI. Glukagon kekal normal dengan EKEE. Penanda stres oksidatif pula menunjukkan EKEE menurunkan MDA secara signifikan, dan meningkatkan TAOC, tetapi tidak signifikan. Keputusan ini mencadangkan keperluan untuk kajian lanjut bagi menilai *E. kingiana* sebagai agen antidiabetik.

IN VIVO ACTIVITIES OF ETHANOLIC EXTRACT FROM *Endiandra kingiana* (LAURACEAE) AS POTENTIAL ANTI-DIABETIC AGENTS

ABSTRACT

Diabetes mellitus is one of the most prevalent diseases in the worldwide population. The impairment in insulin secretion, insulin action or both has led to the hyperglycaemic condition. In 2019, 4.2 million deaths were reported due to diabetes mellitus. Uncontrolled hyperglycaemia condition leads to secondary complications such as retinopathy, nephropathy, cardiomyopathy, and limb amputation. Thus, one of the plants found in Malaysia, known as *Endiandra kingiana* (Pokok Medang) was evaluated. It has *in vitro* α-glucosidase inhibitory effect but lacking evidence in *in vivo* antidiabetic properties. Thus, there is merit to evaluate the antidiabetic effects of oral crude bark *E. kingiana* ethanolic extract (EKEE) on Type 2 diabetic rats (T2DR). The study was divided into acute phase (24 hours) and subacute phase (28 days). T2DR was induced by a combination of high-fat diet (HFD) and low-dose streptozotocin (STZ) (30 mg/kg). A dose of EKEE at 250 mg/kg was found to be most effective in lowering fasting blood glucose (FBS) in the acute study. The effect was further evaluated over 28 days in a sub-acute study. Thirty male Sprague-Dawley (SD) rats were divided into 4 groups. Group 1 – normal, Group 2 – untreated-diabetic rats, Group 3 – diabetic rats on EKEE (250 mg/kg) and Group 4 – diabetic rats on metformin (300 mg/kg). FBS, body weight, BMI and SBP were monitored biweekly. At the end of the study, renal function test, liver function test, lipid profiles, glucagon, and oxidative stress markers were evaluated. EKEE did not significantly reduce FBS. EKEE also did not prevent the reduction of body weight and BMI. Glucagon remains normal with EKEE. As for oxidative stress markers, EKEE significantly decreased

MDA, and increased total antioxidative capacity, but not significant. These results suggested further study are needed to evaluate *E. kingiana* as an antidiabetic agent.

CHAPTER ONE

INTRODUCTION

1.0 Overview

Diabetes mellitus (DM) is one of the chronic diseases where the disease has caused suffering to half a billion people. Fourteen November became a date for global awareness for diabetes or better known as World Diabetes Day, led by the International Diabetes Federation (IDF) and World Health Organization (WHO). “Access to Care” was chosen as the theme for World Diabetes Day for the year 2021 to 2023 (International Diabetes Federation (IDF), 2021). Currently, there are numerous programmes handled by non-government organisations (NGOs) or by international organisation for diabetes awareness in the worldwide population.

Traditional medicine can be defined as the combination of knowledge, skill, and practices based on belief, theories, and experience indigenous to different cultures to prevent, diagnose, and treat physical and mental illnesses (World Health Organization (WHO), 2013). Due to cultural acceptance, compatibility with the human body, and fewer adverse effects, herbal medications are still the mainstay of approximately 75-80 percent of the world population, primarily in underdeveloped countries (Jayaraj, 2010).

Traditional medicine is a practice that existed in society before applying modern science to humans. The usage and acceptance of traditional medicine have been increased significantly throughout the decade. Due to its efficacy and safety, the practices of traditional medicines are passed to next generation. Traditional and complementary medicine (TCM) prevalence usage worldwide ranges from 9.8 to

76.0%, and 69.4% of Malaysian use TCM in their lifetime (Zakaria et al., 2021). The practices include Chinese herbal medicine, acupuncture, moxibustion, *guasha*, cupping, tui-na, natural medicine, homoeopathy, Malay medicine, Malay massage, Ayurveda medicine, chiropractic, reflexology, aromatherapy, and Islamic medicine (Kim, 2017). The herbal plants that are well-known and widely applied in TCM include *Zingiber officinale*, *Murraya koenigii, Syzygium palembanicum, Punica granatum,* which are traditionally used to treat flatulence, dysentery, expel intestinal worms, fever, and headache (Hc et al., 2011). Traditional medicine growth rate in the world market is at 6% annually from 89.9 billion US dollars in 2010 to 114.1 billion US dollars in 2014 based on a report from Global Industry Analyst (Kim, 2017).

The phytochemical constituent in medicinal plants is the secondary metabolites or compounds that treat disease and improve health conditions (Garg et al., 2021). To utilise the compound, investigation is needed to be done from extraction, pharmacological screening, isolation and characterisation, toxicological and clinical evaluation before the compound is safe to be used (Sasidharan et al., 2011). The beneficial compound can be extracted from the fruit, barks, leaves, flower, and plant roots (Karasawa & Mohan, 2018). The increase in demand for traditional medicine has resulted in an action by the Malaysia's Ministry of Health (MOH) by establishing the National Policy on Traditional and Complementary Medicine (TCM) 1999 (Jayaraj, 2010).

1.1 Diabetes Mellitus

Diabetes mellitus (DM) can be defined as a chronic metabolic disorder identified by hyperglycaemia, which caused by insulin secretion impairment, insulin action defect, or both (Punthakee et al., 2018). The word diabetes is originated from Greek means 'siphon', can be explained as to pass through. In Latin, the word mellitus means sweet like honey (Lakhtakia, 2013). The incidence of DM is rising, and according to World Health Organization (WHO, 2016), one in eleven adults has diabetes in the worldwide population. The prevalence of the disease is higher in lower-income and third-world countries without access to medical care.

Additionally, a survey done in Malaysia by the National Health and Morbidity Survey (NHMS) in 2019 reported that 3.9 million adults of 18 years above have diabetes, 9.4% are known diabetes meanwhile 8.9% are undiagnosed (NIH, 2019). In comparison, 8.3% are known for diabetes, meanwhile 5.1% are undiagnosed with diabetes in 2015 (NIH, 2019). DM is a metabolic disease that can be identified by elevation of blood glucose level where prolong occurrence of hyperglycaemia causes tissue damages in the heart, kidney, nerve, eyes, and vascular (Galicia-Garcia et al., 2020). In the study, the positive control used is metformin as the drug is one of the standard drugs used to manage diabetes (Ng et al., 2015). Currently, treatment for diabetes includes injection of insulin and oral hypoglycaemic drugs such as sulfonylureas, biguanides, thiazolidinediones, α-glucosidase inhibitor, incretin agonist, and DPP-4 inhibitors (Lorenzati et al., 2010). Nonetheless, oral hypoglycaemic drugs have side effects such as headache, diarrhoea, nausea, and dizziness (Chaudhury et al., 2017). Thus the alternative method to control the disease, such as natural products, becomes an option due to antioxidant compound properties, contributed by the

presence of phenolics, carotenoids, anthocyanins, and tocopherols (Altemimi et al., 2017). In addition, the possibility of producing a safer anti-diabetic agent increases as the bioactive component help in insulin activity (Patel et al., 2012).

The current study investigated a well-known tree in Malaysia *Endiandra kingiana, also* known as "pokok medang" in Malay, towards the anti-diabetic properties in type 2 diabetic rats. This species is distributed throughout Peninsular Malaysia and Borneo and can be described as medium-sized evergreen trees (Azmi et al., 2014). According to several reports, the family of *Endiandra* shows a few numbers of biological properties such as anti-microbial, anti-cancer, antimalaria, and anti-inflammatory (Abu Bakar et al., 2020). However, there is nominal information regarding its medicinal use in diabetes (Lenta et al., 2015).

1.2 Problem statement

Currently, the conventional diabetic drugs on the market are expensive and have several side effects. Thus, people are more interested in natural product alternatives. Therefore, action needs to be taken to develop new drugs that are safer, cheaper, and effective obtained from medicinal plants to treat and manage diabetes mellitus. Among others, *E. kingiana* has been claimed traditionally to improve blood glucose levels in diabetic people. However, there is a lacking scientific evaluation with regards to the efficacy and safety of *E. kingiana* for diabetes treatment.

1.3 Research questions

Following are the research questions that guided this study:

i. Does *E. kingiana* have an anti-diabetic effect in type-2 induced diabetes rats (T2DM)?

ii. Does the use of *E. kingiana* have any improvement or complication in T2DM?

1.4 Study Objectives

1.4.1 General objective

To evaluate *E. kingiana* bark ethanolic extract (EKEE) as an anti-diabetic agent in type-2 diabetic rats (T2DM).

1.4.2 Specific objectives

i. To evaluate the effect of EKEE on blood glucose level in diabetic rats.

ii. To evaluate the effect of EKEE on body mass index (BMI) in diabetic rats.

iii. To evaluate the effect of EKEE on biochemical parameters (renal function test, liver function test, lipid profile) in diabetic rats

iv. To evaluate the effects of EKEE on oxidative stress markers in diabetic rats

1.5 Research hypotheses

EKEE extract improves the blood glucose level and functions of kidney and liver in diabetic rats.

1.6 Rationale of study

Diabetes mellitus is one of the world's health problems, and cases keep rising every year. The cost of conventional drugs keeps growing due to the demand increases. People choose traditional options like natural products as they are cheaper, easy to obtain and have lesser side effects. The study aims to evaluate the effects of EKEE as an anti-diabetic agent in T2DM rats. This study outcome becomes a basis for a scientific evaluation towards the traditionally claimed of *E. kingiana* towards DM treatment and its safety.

CHAPTER TWO

LITERATURE REVIEW

2.1 Prevalence of Diabetes Mellitus

DM is a chronic disease that affects people all over the world. World Health Organisation (WHO) reported that diabetes directly has caused death to 1.5 million in 2012, and 2.2 million in 2019. Forty-three percent (43%) of 3.7 million deaths occurred under the age of 70. Currently, it is estimated 422 millions adults have diabetes (World Health Organisation, 2016)

Recent IDF Diabetes Atlas reported 4.2 million people had died from diabetes (International Diabetes Federation (IDF), 2019). Moreover, 463 millions of people aged between 20 to 70 years old have diabetes in the year 2019 and predicted to reach 700 million by 2045 (International Diabetes Federation (IDF), 2019). In addition, in the Middle East and North Africa, the world's most populous region, 12.2% of adults aged 20-79 years had diabetes, with 420 thousand deaths in 2019. Moreover, the prevalence estimation of diabetes mellitus in 2030 will rise to 38.8% in this region (International Diabetes Federation (IDF), 2019). The third populous region is the Southeast region, which was reported to have an 8.8% adult population living with diabetes and 1.2 million numbers of deaths in the year 2019. It is estimated in the year 2045, diabetes prevalence in South East Asia will be 152.8 million people.

2.2 Symptoms and complications of diabetes mellitus

Polyuria, polydipsia, weight loss, blurred vision, and genital itch are the common symptoms of new-onset diabetes mellitus. Excess glucose needs to be removed from the body resulting in polyuria and polydipsia. Frequent urination causes a diabetic person to lose a lot of fluid (Type 2 Diabetes Symptoms, 2019). Unexplained weight loss occurs when the body cells lack glucose to use as energy due to insufficient insulin hormone. Thus, the body starts to burn fat and muscle to gain energy, which results in a reduction of overall body weight (Type 2 Diabetes Symptoms, 2019).

The DM complications suffered by the patients have taken their toll through the increased mortality rate. Despite the approved guidelines to control DM, complications are still occurring including diabetic cardiomyopathy, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, and diabetic foot diseases (American Diabetes Association, 2021a)

Diabetic nephropathy is one of the common complications of diabetes that is not detected at the earlier stage. As the disease progresses, the patient becomes symptomatic due to the waste products build-up in the blood. The body cannot get rid of the waste products since the kidneys become less efficient for glomerular filtration, thus resulting in kidney failure (American Diabetes Association, 2021b).

2.3 Diagnosis & classification of diabetes mellitus

Diabetes is a critical disease, which produces a significant effect on the patient's quality of life, morbidity, and mortality. Prevention of complications can be resolved with appropriate treatment and early detection. There are two major types of DM known as Type 1 and Type 2 diabetes, based on the onset and features of the disease. Table 2.1 summarises the criteria for the diagnosis of diabetes mellitus.

According to Centers for Disease Control and Prevention (2021), the patients at risk should need to consider for diabetes screening such as overweight, obese, history with gestational diabetes, a close relative with diabetes, physically inactive or less than 3 times a week.

Table 2.1: Criteria for the diagnosis of diabetes (Adapted from American Diabetes Association, 2021b)

1. In patient with classic symptoms of hyperglycaemia or hyperglycaemia crisis, a random plasma glucose concentration ≥ 11.1 mmol/l. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss. Random is defined as any time of the day.
or
2. FBG ≥ 7.0 mmol/L. Fasting is defined as no caloric intake for at least 8 hours. *
or
3. Two hours post-load glucose ≥ 11.1 mmol/l during an OGTT. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water. *
or
4. HbA1C ≥ 6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the Diabetes Control and Complication Trial assay. *

*In the uncertain hyperglycaemia, diagnosis requires two abnormal test results from the same sample or in two separate test samples.
OGTT, Oral glucose tolerance test
FBG, Fasting Blood Glucose
NGSP, National glycohemoglobin standardisation program

2.3.1 Type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM) generally known as insulin-dependent diabetes or juvenile onset diabetes, requires daily administration of insulin. T1DM is due to ß-cell destruction results in absolute insulin deficiency and represents about 5-10% of all cases of diabetes (Kahanovitz et al., 2017). Several key features of Type 1 DM are shown in Table 2.2.

Table 2.2: Key features of type 1 diabetes mellitus (Adapted from Scobie & Samaras, 2012).

Key Features of Type 1 Diabetes Mellitus
• Insulin treatment is necessary
• Onset is most common in youth, but it can occur at any age
• Positive family history of 10% patients
• 30-50% concordance in identical twins
• Relatively acute onset of disease
• Presence of specific autoantibodies
• Patient is prone to ketosis and associated acidaemia

2.3.2 Type 2 diabetes mellitus

Non-insulin-dependent diabetes mellitus (NIDDM), is also commonly known as type 2 diabetes mellitus (T2DM) which represents 95% of all diabetic cases (American Diabetes Association, 2021a). The main cause of T2DM is due to insulin resistance (IR) and impaired insulin secretion (McCulloch et al., 2016). Insulin resistance in muscle causes increase demand for insulin, meanwhile insulin resistance in the liver cause increase gluconeogenesis and leads to β-cell failure with relative insulin deficiency are several key features of T2DM (Scobie & Samaras, 2012). Table 2.3 summarises the comparison between type 1 and type 2 diabetes mellitus.

Table 2.3: Comparison of type 1 and type 2 diabetes mellitus (Adapted from Mitchell et al., 2006).

Feature	Type 1 DM	TYPE 2
Clinical	Onset: < 20 years, Normal weight, markedly decreased blood insulin.	Onset: >30 years, obese, Increased blood insulin,
Genetics	30-70% concordance in twins, Linkage to MHC class II HLA genes	50-90% concordance in twins, No HLA linkage
Pathogenesis	Autoimmune destruction of ß cells mediated by T cells and humoral mediators (TNF, IL-1, NO)	Insulin resistance in skeletal muscle, adipose tissue and liver, ß-cell dysfunction and relative insulin deficiency.
Islet cell	Insulitis early, marked atrophy and fibrosis	No insulitis, focal insulitis, focal atrophy and amyloid deposition, mild ß-cell depletion

TNF, Tumour necrosis factor
IL-1, Interleukin 1
NO, Nitric oxide

2.4 Diabetes cases in Malaysia

National and Health Morbidity Survey (NHMS) 2019 reported that 3.9 millions adults in Malaysia aged 18 years and above living with diabetes (National Institute of Health (NIH), 2019). Among that, 8.9% of the patients are previously undiagnosed with diabetes. Statistics have shown that the prevalence of diabetes in Malaysia increased up to 18.3% in 2019 compared to the year 2015, which is 13.4% (Ministry of Health, 2020). Apart from that, IDF has reported a total death cases of 22 449 in Malaysia due to DM in the year 2019 (International Diabetes Federation (IDF), 2019).

2.5 Oral hypoglycaemic agent of diabetes mellitus

Currently, there are many types of synthetic antidiabetic drugs available. Nowadays, there are four main groups of oral antihyperglycemic agents (OHA); sulfonylureas, biguanides, thiazolidinediones and α-glucosidase inhibitors (Table 2.4). The typical adverse effects of the OHAs include nausea, vomiting, skin rash, allergies, elevated heart rate, headache, stomach irritation, and lack of appetite (Rupavate, 2014). Furthermore, these hypoglycaemic drugs may cause transplacental passage, resulting in foetal teratogenesis, polycythaemia, hyperbilirubinemia, and hypoglycaemia. (Kavitha et al., 2013). Due to beliefs of natural plants sources to have fewer side effects, more research on antidiabetic drugs made from natural origins (Nabi et al., 2013).

Table 2.4: Classification of oral hypoglycaemic drugs (Adapted from Kavitha et al., 2013)

Groups	Drugs
Sulfonylureas	First generation • Acetohexamide • Chlorpropamide • Tolazamide • Tolbutamide Second generation • Glyburide/glibenclamide • Glipizide • Glimepiride
Biguanides	• Metformin
Thiazolidinediones	• Pioglitazone • Rosiglitazone
Alpha-glucosidase inhibitors	• Acarbose • Miglitol

2.5.1 Metformin

Metformin is a biguanide that is widely used drug due to its safety and low cost. Due to the significant ability to decrease plasma glucose levels, metformin has been used for patients over 60 years to treat T2DM (Lv & Guo, 2020). Rojas et al. (2013) reported that metformin has become the first-line treatment in T2DM due to its efficacy, safety profile, benefit towards cardiovascular and metabolic effects, and its capacity to be combined with other antidiabetic agents.

Metformin suppresses gluconeogenesis and increases glucose and insulin sensitivity in peripheral tissue for its antihyperglycemic effect (Correia et al., 2008). The drug inhibits mitochondrial complex I, which then reduces the production of adenosine triphosphate (ATP) and intracellular adenosine diphosphate (ADP). The cellular level of adenosine monophosphate (AMP) increases, thus leading to the activation of AMPK (Lv & Guo, 2020). Metformin also activates AMP-activated protein kinase (AMPK) in hepatocytes, thus suppressing glucose production, normalising blood glucose level and stimulate glucose uptake (Figure 2.1). In addition, metformin suppresses protein synthesis and gluconeogenesis through activation of AMPK in rapamycin (TOR) pathway (Correia et al., 2008). Furthermore, metformin-induced AMPK activation led to inhibition of hepatic lipogenesis, which stimulates liver fatty acid oxidation. Later, the stimulation causes the correction of dyslipidaemia and improves insulin resistance (Zheng et al., 2015).

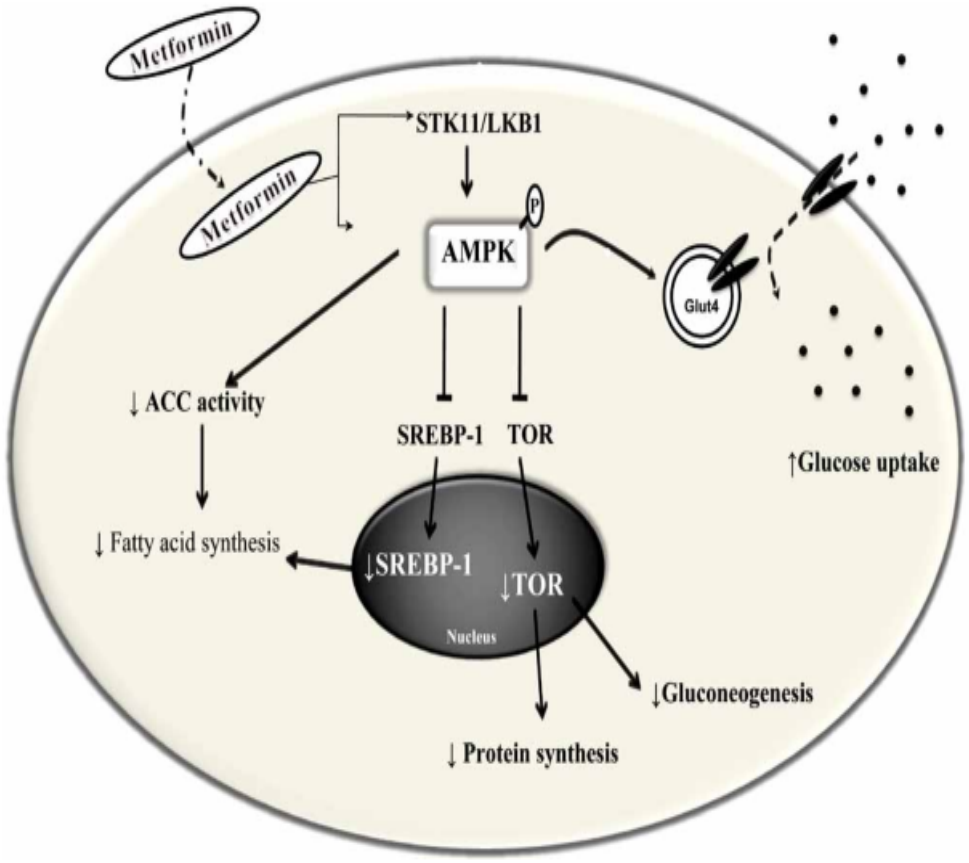

Figure 2.1: Mechanism of action metformin (Adapted from Correia et al., 2008).

2.6 *Endiandra kingiana*

Endiandra kingiana or "pokok medang" in Malay, was evaluated in this study as a basic scientific evaluation towards diabetes treatment due to no information regarding its medicinal use on diabetes (Lenta et al., 2015). This plant belongs to the species of *Endiandra*. *E. kingiana* belongs to the family known as Lauraceae (Azmi et al., 2014).

2.6.1 Biological activities of *E. kingiana* extract

The family of *Endiandra* shows a few numbers of biological properties such as anti-microbial, anti-cancer, antimalaria, and anti-inflammatory (Abu Bakar et al., 2020). Endiandric acid derivatives have been found in 4 species of Endiandra (*Endiandra baillonii, E. introsa, E. jonesii,* and *E. kingiana*). The structural properties that are common are the cyclic nature, double bonds and terminal carboxylic acid groups and the compound can be grouped based on the carbon skeleton layout. The first group is characterised with thirteen carbon atom fused tetracyclic ring system, second group with eleven atom fused tetracyclic ring system and the last group with bi-, tri, or tetracyclic system besides first and second skeleton (Lenta et al., 2015). The study of the methanolic extract of *Endiandra kingiana* bark led to the isolation of endiandric acid analogs kingianic acids F, G and endiandric acid (Azmi et al., 2014). Azmi et al. (2016) also isolated Kingianic acids A–E with phenylalkyl side chains from the bark of *E.kingiana,* which further led to the isolation of a series of polyketides as a racemic-mixtures, which having each an amide function and named kingianins A–N. The study then identify the binding affinity of the racemic mixtures of kingianin A–N isolated from E. *kingiana,* evaluated on Bcl-xL by competition against the fluorescently tagged BH3 domain of the protein Bak. Racemic mixtures of kingianins

G–L exhibited good binding affinity with kingianin G, exhibiting the best potency with a Ki value of 2 ± 0 μM.

Subsequently, kingianic acids A, C, E, F, G, endiandric acid M, and tsangibeilin B isolated from *E. kingiana* were evaluated for their cytotoxicity activity against A549 (lung adenocarcinoma epithelial cell lines), HT29 (colorectal adenocarcinoma cell lines) and PC3 cell lines. All compounds show were no activity against prostate adenocarcinoma cancer cell lines. Kingianic acid A showed weak activity against HT-29 and A549 cell lines with IC50 values of 35 ± 0.2 μM and 85.4 ± 0.2 μM, respectively. Kingianic acid E showed moderate cytotoxic activity against A549 and HT-29 cell lines with IC50 values of 15.36 ± 0.19 μM and 17.10 ± 0.11 μM, respectively (Lenta et al., 2015). This was the first report of the cytotoxicity of this class of secondary metabolites.

In the study of potential inhibitors for dengue type 2 NS2B/NS3 serine protease, the phytochemical investigation of *E. kingiana* bark extract produced a new benzofuranone compound that was isolated, 4-hydroxy-6-(9,13,17-trimethyldodeca-8,12,16-trienyl)-2(3H)-benzofuranone, together with 6 known compounds (−)-epicatechin, (+)-catechin, methyl orsellinate, vanillic acid, vanillin, and cinnamtannin B1. *In-vitro* study was carried out to test NS2B/NS3 protease of DENV2 with 4-hydroxy-6-(9,13,17-trimethyldodeca-8,12,16-trienyl)-2(3H)-benzofuranone, (−)-epicatechin, (+)-catechin, methyl orsellina and the crude extract of E. kingiana. Compound 4-hydroxy-6-(9,13,17-trimethyldodeca-8,12,16-trienyl)-2(3H)-benzofuranone, (−)-epicatechin, (+)-catechin had moderate activity with more than 60%

inhibition. This shows that Endiandra species possessing dengue inhibitors (Sulaiman et al., 2019).

The preliminary screening on major compounds from *E. kingiana*; kingianins A and F showed appreciable α-glucosidase inhibitory effect in a concentration-dependent manner (IC50 = 19.7 ± 2 and 11.9 ± 2, respectively) (Azmi et al., 2014).

2.7 Streptozotocin (STZ)

The study used diabetic rats induced by streptozotocin (STZ). STZ is a chemical compound derived from *Streptomyces achromogenes* that is used clinically as a chemotherapeutic agent in the treatment of pancreatic β cell carcinoma (Graham et al., 2011) (Figure 2.2). STZ was first reported to have diabetogenic properties in 1963. The STZ-induced diabetic animal models have been beneficial in identifying the mechanisms of diabetic pathogenesis and in screening artificial chemicals, natural products, and pharmacological agents that are potentially capable of lowering blood glucose levels (Wu & Yan, 2015). In addition, STZ has been used for induction of T1DM or T2DM in animals. A single STZ injection in rodents can induced T1DM model. For T2DM model, there are three approaches in induction to diabetes; which include administration of nicotinamide followed with STZ injection, high-fat diet (HFD) feeding a low-dose STZ injection, and STZ injection during the neonatal period (Wu & Yan, 2015). In the pancreas, STZ is capable of inducing selective destruction towards beta cells resulted in causing a state of insulin-dependent DM (Lenzen, 2007).

Figure 2.2: Structure of Streptozotocin (Lenzen, 2007).

STZ has a short half-life as the compound is rapidly metabolised and eliminated by the renal. The STZ induces acute toxicity to the liver and kidney but is negligible after the hyperglycaemia becomes persistent (Wu & Yan, 2015). The mechanism of STZ toxicity on ß-cells is illustrated in Figure 2.3. Through glucose transporter (GLUT2), STZ enters the ß-cell, resulting in the production of nitric oxide (NO), superoxide anions (O_2), hydroxyl radicals ($HO^{\cdot}$), and peroxynitrite ($ONOO^-$) led to DNA alkylation. Prolonged DNA alkylation led to DNA damage and inhibition of mitochondrial function (Šoltésová & Herichová, 2011).

The massive DNA damage resulting overstimulates poly (ADP-ribose) polymerase, which later causing NAD^+ depletion with subsequent ATP deficiency. STZ also inhibits N-acetyl-β-D-glucosaminidase led to the accumulation of irreversible glycosylated proteins. β-cell destruction occurs due to the accumulation as time progressed, thus resulting in a condition of insulin-dependent diabetes (Šoltésová & Herichová, 2011).

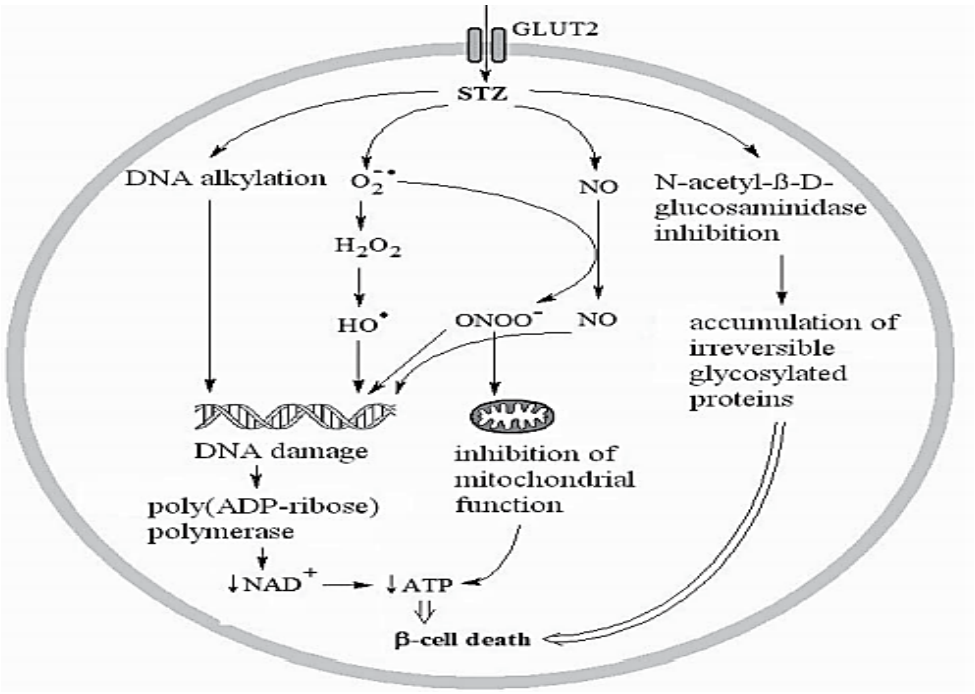

Figure 2.3: Schematic representation of intracellular processes contributing to streptozotocin (STZ) toxicity (Šoltésová & Herichová, 2011)

2.8 Medicinal plants with antidiabetic properties

Plants have been a source of treatment, and many drugs either derived directly or indirectly and being used currently worldwide for diabetes treatment. Currently, there are estimated about 800 plants possess anti-diabetic potential in treatment for T2DM (Patel et al., 2012). Table 2.5 listed out several medicinal plants that have been studied and shown potential anti-diabetic properties.

Table 2.5: Several plants with antidiabetic properties

Plants	Parts	Studies
Trigonella foenum-graecum)	Seed	(Swaroop et al., 2014)
Catharanthus roseus	Leaf	(Ohadoma & Michael, 2011)
Cucumis trigonus	Fruit	(Salahuddin & Jalalpure, 2010)
Camellia sinensis	Leaves	(Islam, 2011)
Emblica officinalis	Fruit	(Elobeid & Ahmed, 2015)
Eugenia jambolana	Seed	(Sridhar et al., 2005)

2.9 Animal models for diabetes

A substantial amount of research has been done on an animal model of DM. Animal models play an important role in the exploration and characterisation of disease pathophysiology, target identification and the evaluation of novel therapeutic agents and treatments *in vivo* particularly in DM (Al-awar et al., 2016). Animal models for T2DM can be designed in both obese and non-obese animal models with several degrees of insulin resistance and ß-cell failure. The animal model for T1DM can range from animals with spontaneously developing autoimmune diabetes to ablation of the chemical of the pancreatic beta cells (King, 2012). An experimental animal model to mimic T2DM induces animal model with two stressors, HFD and treatment by ß-cell toxin STZ. The experimentally-induced model resulted in a reduction of functional ß-cell, which characterise T2DM (Skovsø, 2014).

CHAPTER THREE

MATERIALS AND METHODS

This study begins with the preparation of ethanolic extraction of *Endiandra kingiana* and followed by the animal study. The overall flowchart of this study is shown in Figure 3.1.

3.1 Plant Materials

E. kingiana used in this study was collected from Kuala Lipis Forest, Pahang, Malaysia that was identified by T. Leong Eng and voucher number were identified and deposited in the herbarium of Department of Chemistry, University of Malaya, Kuala Lumpur. The bark of *E. kingiana* was selected for extraction by our collaborators at Universiti Malaysia Terengganu. Figure 3.2 and Figure 3.3 show the image of the plant.

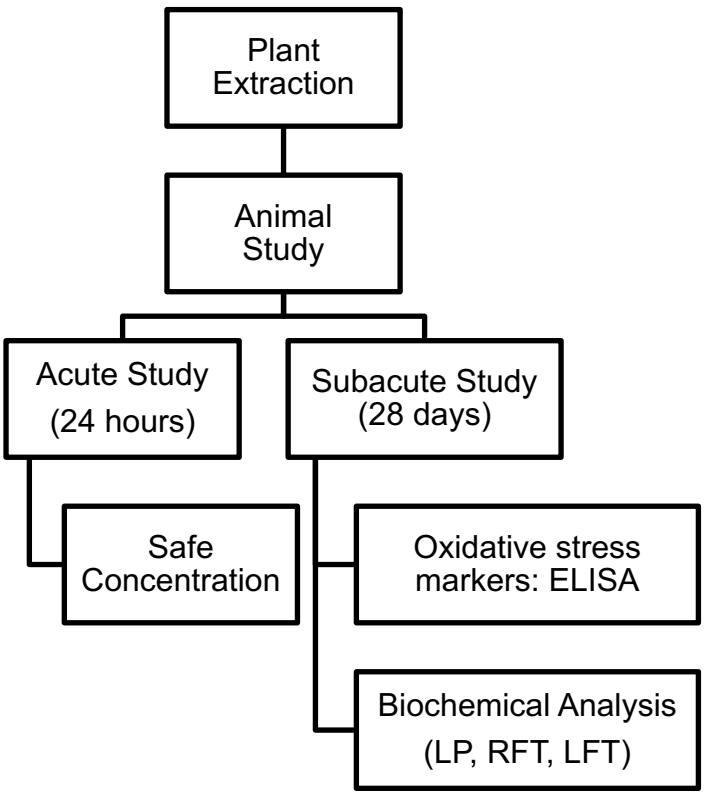

Figure 3.1 Overall flowchart of the study